



Ground Water Issue

Microbial Processes Affecting Monitored Natural Attenuation of Contaminants in the Subsurface

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Introduction

The EPA Regional Ground Water Forum is a group of EPA professionals representing Regional Superfund and Resource Conservation and Recovery Act (RCRA) Offices, committed to the identification and resolution of ground-water issues impacting the remediation of Superfund and RCRA sites. Innovative technologies for subsurface remediation are being evaluated more often for specific sites, as the limitations to conventional technologies are recognized. The purpose of this Issue Paper is to provide those involved in assessing remediation technologies with some basic information regarding monitored natural attenuation (MNA) processes, specifically in determining overall contribution of microbial processes.

On April 21, 1999, the Office of Solid Waste and Emergency Response (OSWER) issued Directive 9200.4-17P, titled "Use of Monitored Natural Attenuation at Superfund, RCRA Corrective Action, and Underground Storage Tank Sites." It specifies the current EPA policy regarding the use of MNA for the remediation of contaminated soil and ground water at OSWER sites. The Directive's aim is to promote consistency in the manner in which MNA remedies are proposed, evaluated, and approved. Accordingly, "EPA does not consider MNA to be a presumptive or default remedy – it is merely one option that should be considered with other applicable remedies." Thus, during the process of selecting a site remedy, MNA may be evaluated and compared with other remedial technologies and chosen upon attainment of each relevant remedy selection criteria, including the full protection of human health and the environment, and achieving the intended site remedial objectives within a time frame that is comparable to the other remedial techniques. The Directive expects that "source control and long-term performance monitoring will be fundamental components of any MNA remedy" (U.S. EPA, 1999).

It should be emphasized that this document is not intended to be used in establishing protocol involved in natural attenuation investigations or policies leading to the interpretation of the results of those investigations. To that end, the reader is referred to the EPA Protocol for chlorinated solvents (U.S. EPA, 1998a), and ASTM for petroleum hydrocarbons (ASTM, 1998). EPA is also preparing a guidance for long term monitoring (LTM) for MNA.

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Background

Interest in the natural attenuation of ground-water contaminants has increased in recent years as the result of dealing with complexities of subsurface systems and the inherent problems and costs associated with more conventional remedial technologies, such as pump-and-treat systems. There is a growing perception in the environmental community that, under favorable conditions, the selection of natural attenuation as a remedy will result in significant savings in cost over more intrusive remedial alternatives which are exacerbated by the complex geochemical, biochemical, and hydrogeological uncertainties which are dominant at most sites. While this perception may or may not be true, depending upon site specific

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characteristics, like all relatively new technologies, actual cost and performance data will be required as the remediation alternative matures. Natural attenuation, which is also referred to as natural assimilation, intrinsic remediation, intrinsic bioremediation, natural recovery, or passive remediation, is the use of natural processes to remove contaminants from soil or ground water. While the mechanisms of chemical transformation, dispersion, dilution, sorption and volatilization have been identified, aerobic and anaerobic degradation comprise the major processes for the reduction of contaminant mass in the subsurface. Some considerations needed for the evaluation of these processes are depicted in Table 1.

There is little question that naturally occurring biodegradation processes are taking place at many sites where sediments have been contaminated (Davis et al., 1994; Lee, 1988). To those who have examined subsurface sediment, the reduced mineralogy (darkening) and unpleasant odors (anaerobic metabolic products) are indicators of microbial degradation processes. While there are limitations to natural attenuation due to factors such as complex hydrogeology, microbial toxicity of contaminants, and other physical, biological, chemical and environmental factors, many of the organic compounds introduced into the subsurface can be transformed by indigenous microorganisms. The primary challenge in evaluating natural attenuation is not in demonstrating that biodegradation is

occurring. This can be a relatively easy task accomplished by determining that the production of metabolites and the loss of electron acceptors are concomitant with the distribution of contaminants. As with any other remedial technology, the appropriate evaluation of natural attenuation as a remedial alternative is to make the determination that the transformation processes are taking place at a rate that is protective of human health and the environment. The evaluation should include a reasonable expectation that these processes will continue at an acceptable rate for an acceptable period of time.

Chlorinated aliphatics are among the most widespread contaminants in ground water (Figure 1) and soil due to their use for degreasing, dry cleaning, and as solvents (Westrick et al., 1984). At the same time, the impact of petroleum hydrocarbons among the various environmental media is ubiquitous (Tiedje and Stephens, 1988; Sharples, 1992; Kennedy and Hutchins, 1992). Because of their prominence as environmental contaminants, these two groups of chemicals will be used as case examples in this issue paper.

Monitored Natural Attenuation Model

Contaminants in the subsurface partition into four phases including sorption to the soil and aquifer solids, a free phase which displaces water from the pore spaces, dissolved in the

Table 1. Some Information Needed for Prediction of Organic Contaminant Movement and Transformation in Ground Water. (Reprinted from *Biotic and Abiotic Transformation of Halogenated Aliphatic Compounds*, T.M. Vogel, Ph.D. Thesis, Stanford University, Stanford, CA, p. 42, 1988, with permission of T.M. Vogel.)

| BIOLOGICAL | Ground-Water Characteristics | Aquifer Characteristics | Contaminant Characteristics |
|-------------------|--|--|---|
| | ionic strength pH temperature nutrients substrate O_2 , NO_3^- , $SO_4^{=}$ macro (P, S, N) trace organism | grain size active bacteria number Monod rate-constants | potential products toxicity concentration |
| CHEMICAL | Ground-Water Characteristics | Aquifer Characteristics | Contaminant Characteristics |
| | ionic strength pH temperature NO_3^- , $SO_4^{=}$, O_2 toxicants | potential catalysts metals, clays | potential products concentration |
| HYDRAULIC | Contaminant Source | Wells | Hydrogeologic Environment |
| | location amount rate of release | location amount depth pump rates | extent of aquifer and aquitard characteristics of aquifer hydraulic gradient ground-water flow rate |
| SORPTION | Distribution Coefficient | Characteristics of the Aquifer Solid | Contaminant Characteristics |
| | characteristic of concentration | organic carbon content clay content | octanol/water partition coefficient solubility |

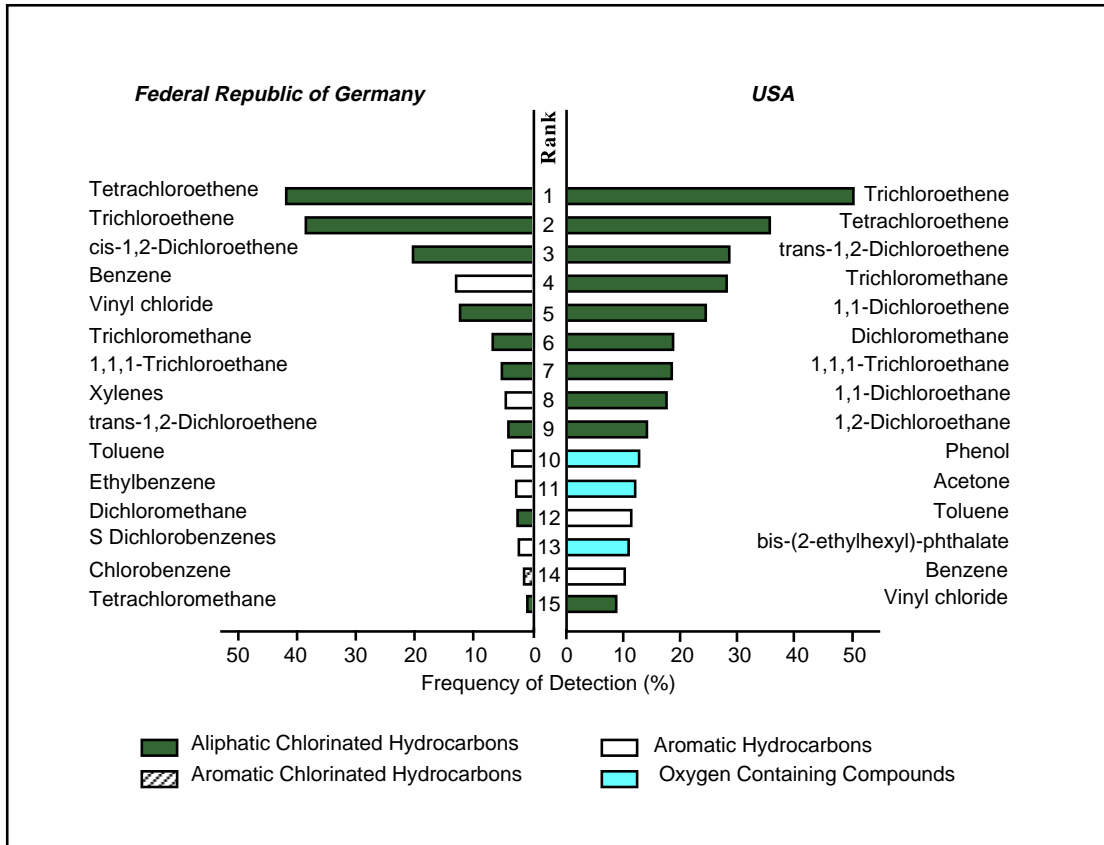


Figure 1. The 15 most frequently detected organic compounds in groundwater at waste disposal sites in Germany and the U.S. (Reprinted from *The Landfill*, Baccini, P., Ed., p. 399, 1989, Arneht, J.-D., Milde, G., Kerndorff, H., and Schleyer, R. with permission of Springer-Verlag, New York, Inc., New York, NY.)

water, and vapor (Figure 2). The degree to which contaminants partition into these phases is determined by their physical/chemical properties or notably the sorption coefficient, Henry's Law Constant (gas partition coefficient), octanol-water coefficient,

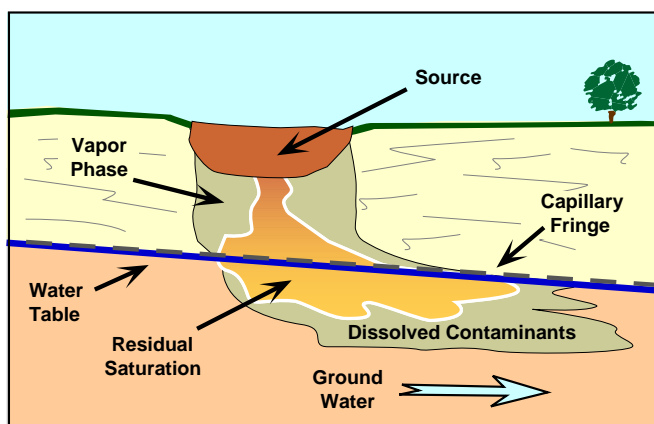


Figure 2. Distribution of contaminants in the subsurface. (Modified from *In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies*, p. 2, 1993, by Norris et al., EPA/600/R-93/124.)

and solubility (water partition coefficient). The mass in each phase will therefore depend on the preference of the contaminants of concern for each phase.

Plume movement is dependent on the same partition coefficients, in addition to the hydrology of the site itself. In most instances, chemicals will prefer to partition to organic matter within sediments, to oily or free-phase material that may completely fill pore spaces, or be dissolved in water at solubility levels. The mass of the contaminants in the aqueous phase is usually lower than that in the other phases.

As water moves through pore spaces, chemicals are desorbed from sediments or dissolved from free-phase liquids. Once in solution, these chemicals move with the aqueous phase. Their movement downgradient is impeded by further sorption to sediments and biodegradation. The basis of natural attenuation is that the partition of chemicals into the aqueous phase reaches equilibrium with the processes of biological transformation at an acceptable time and distance from the source. To understand these biological processes, some knowledge of microbiology is required.

Microbial Physiology

Bacteria, although unicellular, share characteristics with most living things. Information is encoded in DNA (deoxyribonucleic

acid) and transferred through RNA (ribonucleic acid) to ribosomes to make proteins or enzymes which are used to operate systems within the organism. In regard to this discussion, enzymes are responsible for the degradation of organic carbon, which is used by the bacterial cell to produce both the building blocks of life and energy. The degradation of any organic molecule, including contaminants, requires the production and efficient utilization of enzymes. In most instances, degradation is merely a complex oxidation/reduction reaction. The electrons or reducing equivalents (hydrogen or electron-transferring molecules) produced must be transferred to a terminal electron acceptor (TEA). During the transfer process, energy is produced which is utilized by the cell.

In regard to TEAs, bacteria are generally grouped into three categories:

1. *Aerobic bacteria* — Bacteria which can only utilize molecular oxygen as a TEA. Without molecular oxygen, these bacteria are not capable of degradation.
2. *Facultative aerobes/anaerobes* — Bacteria which can utilize molecular oxygen or when oxygen concentrations are low or nonexistent, may switch to nitrate, manganese oxides or iron oxides as electron acceptors.
3. *Anaerobes* — Bacteria which cannot utilize oxygen as an electron acceptor and for which oxygen is toxic. Though members may utilize nitrate or other electron acceptors, it can be said that they generally utilize sulfate or carbon dioxide as electron acceptors.

In this discussion, three modes of contaminant degradation are addressed including aerobic, cometabolic, and anaerobic. The first is the case in which the contaminants, for example, petroleum hydrocarbons, are utilized by bacteria as a sole source of carbon. Petroleum hydrocarbons are degraded through a series of enzymatic reactions to produce needed cellular constituents. Electrons or reducing equivalents must be regenerated. If a contaminant serves as a sole source of carbon and energy, conditions must be within acceptable pH, Eh, and temperature limits and the appropriate TEA must be present. In this case, the rate of degradation will be determined by the rate of dissolution of toxic end products away from the microbial population and the rate at which the TEA is replenished. Some of the lesser chlorinated solvents, such as dichloroethene (DCE), may also serve as sole sources of carbon; however, tetrachloroethene (PCE) and trichloroethene (TCE) are not thought to serve as sole sources of carbon.

In the case of TCE (and lesser chlorinated solvents), degradation may occur through cometabolic processes. Under aerobic conditions, the enzymes necessary for the degradation, however, must be induced. Inducible enzymes are those that are not produced unless an inducer compound is present within the bacterial cell. Pertinent to this discussion are the inducers for methane monooxygenase and various mono- and dioxygenase enzymes produced by aromatic degrading bacteria.

In the presence of oxygen and methane, methanotrophic bacteria are known to produce the enzyme methane monooxygenase (Hanson and Hanson, 1996; Patel et al., 1982). The substrate for this enzyme is methane, but it has been shown to have a broad substrate specificity including chlorinated solvents (Mayer et al., 1988). Methanotrophs downgradient from a chlorinated solvent event may feed on methane produced within the

anaerobic portion of the plume and cometabolically degrade some chlorinated solvents.

Numerous authors have shown that oxygenase enzymes produced by bacteria capable of degrading aromatic hydrocarbons are capable of degrading chlorinated solvents. Aromatic compounds, such as toluene and phenol, have been shown to induce the responsible enzymes. In contaminated aquifers which contain both aromatic hydrocarbons and chlorinated solvents, degradation of both may occur.

It should be noted that reports on the degradation of PCE under aerobic conditions do not exist in the peer-reviewed literature. The structure and oxidative state of PCE may preclude its aerobic degradation (Chen et al., 1996). The anaerobic process for degradation of chlorinated solvents is known as reductive dechlorination (Bouwer et al., 1981; Bouwer, 1994). Chlorinated solvents are not utilized as a carbon source, rather as an acceptor for electrons produced during the metabolism of other oxidizable carbon (electron donor). Thus, this process cannot be termed cometabolism. Electrons or reducing equivalents formed during metabolism are accepted by the chlorinated solvent. As an example, PCE will accept electrons or reducing equivalents formed during metabolism. This results in the reduction of PCE, the concomitant release of a chloride ion and the formation of TCE. While almost any degradable carbon appears capable of driving reductive dechlorination, in most instances, a low percentage of the electrons or reducing equivalents are utilized in the reductive process. This is a function of other metabolic requirements for reducing equivalents (substrate specific) and the presence or absence of more suitable TEAs.

The behavior of the chlorinated solvent plumes can be classified into three types based on their primary substrate source (U.S. EPA, 1998a).

- *Type 1 Behavior* — Primary substrate is adequate amount of anthropogenic organic carbon, solvent plume degrades
- *Type 2 Behavior* — Primary substrate is adequate amount of native organic carbon, solvent plume degrades
- *Type 3 Behavior* — Low native organic carbon concentrations or low anthropogenic organic carbon concentrations, PCE, TCE, and DCE do not degrade

For complete detoxification, the parent chlorinated solvent must be dechlorinated in a stepwise fashion to the environmentally benign ethene. This is illustrated in Figure 3. While PCE and TCE are readily reduced as a result of their oxidative states, the more reduced daughter products (DCE and VC) are less prone to reductive processes. These intermediates tend to accumulate in anaerobic aquifers where contaminants are allowed to naturally attenuate (Lesage et al., 1990). While this may indeed be a function of the oxidative state of the lesser-chlorinated compounds, it may also be a function of the concentration of degradable organic matter within the contaminated system.

The efficacy of an anaerobic microbial population for natural degradation of chlorinated solvents is determined by the same environmental factors as for the other systems (i.e., pH, Eh, temperature, osmotic potential), the presence of a carbon source which can be readily degraded (electron donor), and a

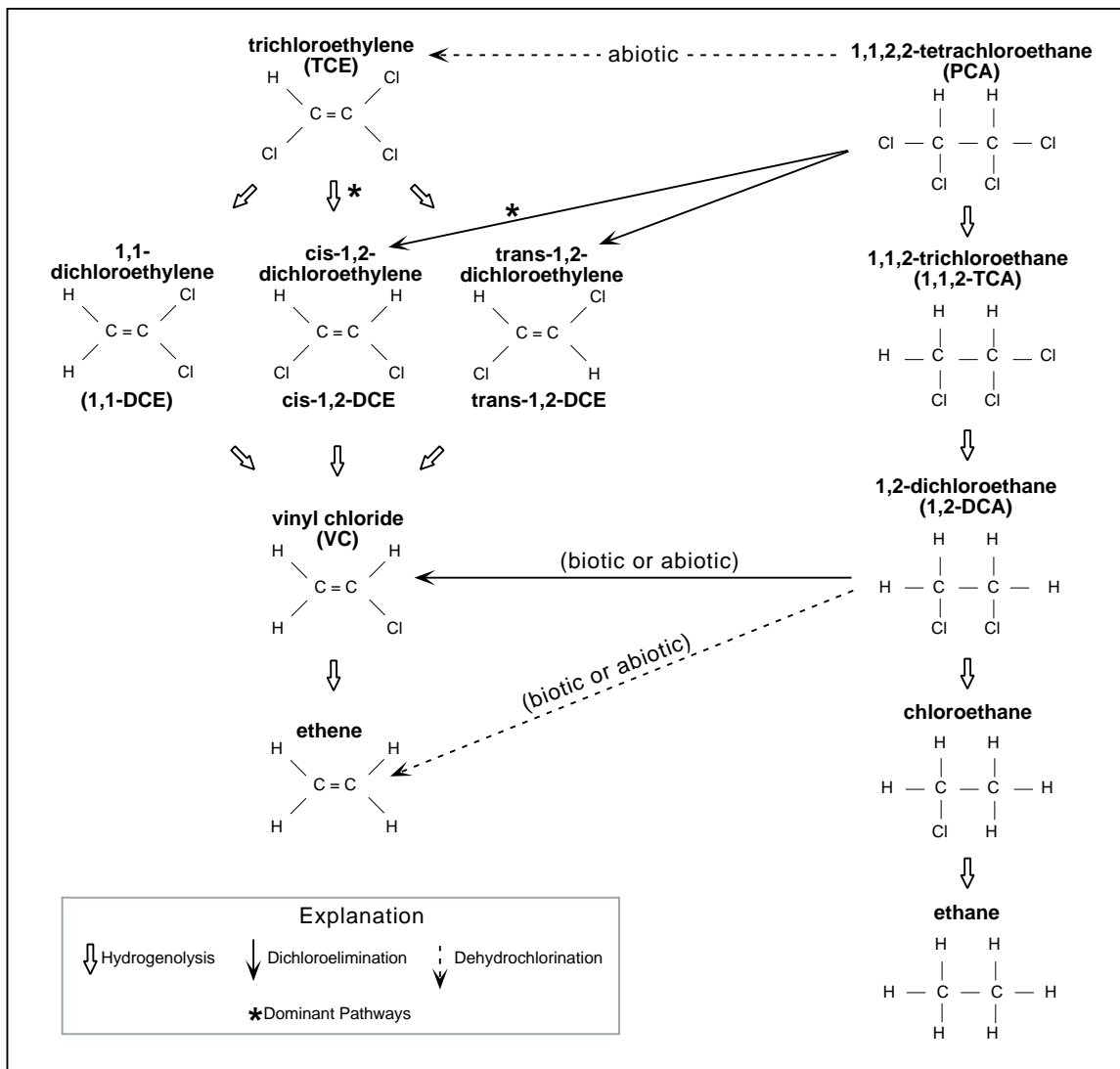


Figure 3. Anaerobic degradation pathways for trichloroethylene and 1,1,2,2-tetrachloroethane. (Modified from Chen et al., 1996; McCarty, 1996; Nyer and Duffin, 1997; and Vogel et al., 1987.)

TEA other than chlorinated solvents (Vogel et al., 1987). In addition to organic carbon and a TEA, bacteria require macro- and micronutrients, most notably nitrogen and phosphorous, for the production of DNA, RNA and other needed cellular constituents. In most instances within an aquifer, sufficient macro- and micronutrients will be available for microbial processes. On the other hand, the availability of organic carbon can often be the limiting factor in the continuance of these processes. For example, during the reductive dechlorination of chlorinated solvents, the ratio of the mass of the electron donor to that of the contaminant ranges between 100/1 and 1000/1 (Bouwer, 1994).

Mechanisms of Biodegradation

In theory, in any environment in which microbial activity occurs, there is a progression from aerobic to anaerobic (methanogenic)

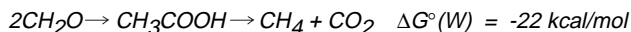
conditions. There is a definite sequence of electron acceptors used in this progression through distinctly different redox states (Figure 4).

The rate, type of active microbial population, and level of activity under each of these environments are controlled by several factors. These include the concentration of the electron acceptors, substrates which can be utilized by the bacteria, and specific microbial populations leading to the progression of an aquifer from aerobic to methanogenic conditions (Salanitro, 1993). This results in a loss of organic carbon and various electron acceptors from the system as well as a progression in the types and physiological activity of the indigenous bacteria.

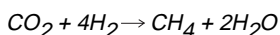
If microbial activity is high, the aquifer environment would be expected to progress rapidly through these conditions. The following scenario outlines a general sequence of events in which

Conversion of organic matter, represented by the model compound CH_2O , in different redox environments may be represented by the following stoichiometric reactions and the corresponding Gibbs free-energy changes at pH equal to 7.

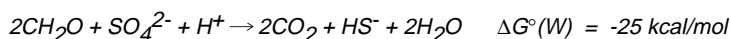
Methanogenic, fermentative:



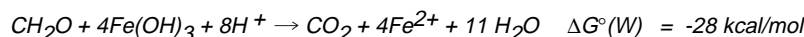
Note: For organic matter deviating from the used model compound, the fermentation will lead to generation of H_2 , which may be oxidized by CO_2 reduction according to:



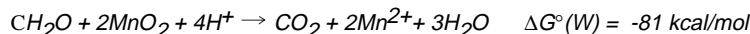
Sulfate reduction:



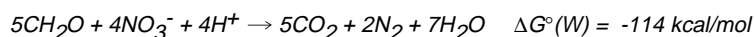
Iron (ferric) reduction:



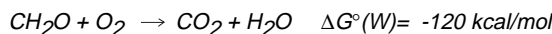
Manganese (manganic) reduction:



Denitrification:



Aerobic respiration, oxygen reduction:



These processes are microbially mediated.

Figure 4. Examples of redox reactions involved in degradation of organic matter (expressed as the model compound CH_2O) in different redox environments. (Reprinted from *Attenuation of Landfill Leachate Pollutants in Aquifers*, box 1, p. 138, 1994, by Christensen, T.H., P. Kjeldsen, H.-J. Albrechtsen, G. Heron, P.H. Nielsen, P.L. Bjerg, and P.E. Holm, with permission of CRC Press, Inc., Boca Raton, FL.)

aerobic metabolism of preferential carbon sources would occur first. The carbon source may be contaminants of interest or other more readily degradable carbon which has entered the system previously or simultaneously with the contamination event.

Oxygen-Reducing to Nitrate-Reducing Conditions

Once available oxygen is consumed, active aerobic populations begin to shift to nitrate respiration. Denitrification will continue until available nitrate is depleted, or usable carbon sources become limiting.

Nitrate-Reducing to Manganese-Reducing Conditions

Once nitrate is depleted, populations which reduce manganese may become active. Bacterial metabolism of substrates utilized by manganese-reducing populations will continue until the concentration of manganese oxide becomes limiting.

Manganese-Reducing to Iron-Reducing Conditions

When manganese oxide becomes limiting, iron reduction becomes the predominant reaction mechanism. Available evidence suggests that iron reduction does not occur until all manganese IV oxides are depleted. In addition, bacterial Mn(IV) respiration appears to be restricted to areas where sulfate is nearly or completely absent.

Iron-Reducing to Sulfate-Reducing Conditions

Iron reduction continues until substrate or carbon limitations allow sulfate-reducing bacteria to become active. Sulfate-reducing bacteria then dominate until usable carbon or sulfate limitations impede their activity.

Sulfate-Reducing to Methanogenic Conditions

Once usable carbon or sulfate limitations occur, methanogenic bacteria are able to dominate.

The ambient redox condition of the aquifer is important when determining the contribution of microbial degradation to MNA mechanisms. In the case of petroleum hydrocarbons, because of their highly reduced condition, the preferred TEA for microbial processes would be oxygen (Brown et al., 1996; Clark et al., 1997). From a thermodynamic standpoint, this is the most favorable reaction mechanism. When the soluble portion of petroleum hydrocarbons, BTEX (benzene, toluene, ethylbenzene, xylenes), are the contaminants of concern, an inverse relationship between BTEX and dissolved oxygen concentrations within a plume is indicative of the microbial metabolism of these compounds as well as other hydrocarbons in the mixture (Donaldson et al., 1992; Huesemann and Truex, 1996). Data available from various sites indicate that the natural attenuation of BTEX proceeds at higher rates under oxic conditions than normally achieved in anoxic environments (Figure 5), with rate constants ranging from 0.3 to 1.3 percent per day when modeled as a first-order process (Chiang et al., 1989; Kemblowski et al., 1987; Salanitro, 1992; and McAllister and Chiang, 1994). Although anaerobic biodegradation of toluene and xylene under nitrate-reducing (Barbaro et al., 1992; Schocher et al., 1991), iron-reducing (Lovley, et al., 1989; Lovley and Lonergan, 1990), sulfate-reducing (Beller et al., 1992a and b; Edwards and Grbić-Galić, 1992; Rabus et al., 1993), and methanogenic (Vogel and Grbić-Galić, 1986; Bouwer and McCarty, 1983; Edwards and Grbić-Galić, 1994) conditions have been extensively reported. Until recently, unequivocal biodegradation of benzene under strict anaerobiosis was not demonstrated (Edwards and Grbić-Galić, 1992; Lovley et al., 1994). According to Borden et al. (1997), even though accurate description of anaerobic biodegradation of individual BTEX constituents may not follow a simple first-order decay function, biodegradation of total BTEX seems to more closely approximate a first-order decay function.

Biodegradation of chlorinated solvents, depending on the degree of halogenation (Figure 6), is fundamentally different from that of petroleum hydrocarbons and other oxidized chemicals (Wiedemeier et al., 1995). The preferred redox conditions for the effective degradation of these chemicals is anaerobic (exception is vinyl chloride, VC). Effective degradation of these compounds may occur only when redox conditions are below nitrate reducing.

Although under aerobic conditions, cometabolism of TCE by autotrophic bacterial populations, obtained from soil and ground water, have been demonstrated, it is generally accepted that

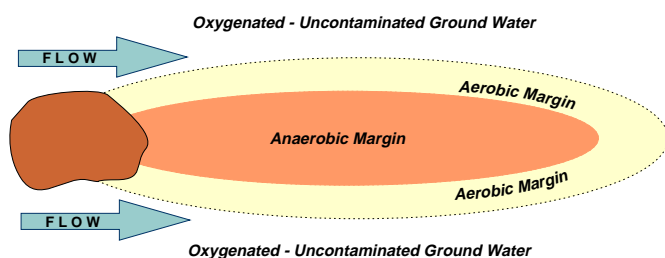


Figure 5. Plan view of a typical hydrocarbon plume undergoing natural attenuation. (Modified from *In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies*, p. 9-8, 1993, by Norris et al., EPA/600/R-93/124.)

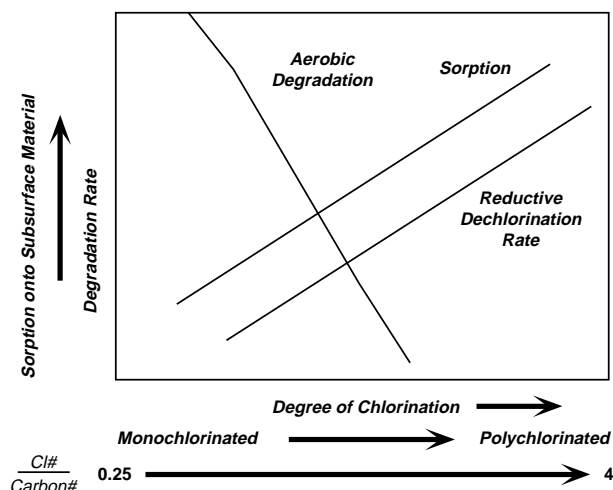


Figure 6. Relationships between degree of chlorination and anaerobic reductive dechlorination, aerobic degradation and sorption onto subsurface material. (Reprinted from *In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies*, p. 10-19, 1993, by Norris et al., EPA/600/R-93/124.)

this route of removal is limited only to low concentrations of TCE. The cometabolism of TCE proceeds in the presence of methane (Fogel, et al., 1986; Henson et al., 1988; Wilson and Wilson, 1985), ammonia (Arciero et al., 1989), or toluene (Ensley, 1991; Mu and Scow, 1994; McCarty and Semprini, 1994) as cosubstrate. Due to the inherent toxicity of TCE to microorganisms responsible for degradative process (Alvarez-Cohen and McCarty, 1991a; Broholm et al., 1990), and because of the competitive inhibition between a cosubstrate and the secondary substrate for oxygenase enzymes (Alvarez-Cohen and McCarty, 1991b; Hopkins et al., 1993), special attention to concentrations of TCE and its cosubstrate is warranted. Toxic or inhibitory effects of TCE are more serious than those of 1,1,1-TCA (Broholm et al., 1990).

Microcosm studies involving anaerobic biotransformation of PCE/TCE from environmental samples including sediments (Parsons et al., 1984), ground water (Wilson et al., 1983; Sewell and Gibson, 1991), and soil (Kleopfer et al., 1985) have been documented. Also, the reductive dechlorination of PCE and other chlorinated compounds under methanogenic conditions has been reported (Vogel and McCarty, 1985 and 1987b; Vogel et al., 1987; Freedman and Gossett, 1989; McCarty, 1988). Bagley and Gossett (1990) suggested that the ability of sulfate-reducing enrichment cultures for PCE dechlorination is apparently less than that of mixed methanogenic cultures. The main by-product of anaerobic biodegradation of chlorinated ethene is VC which is more toxic than the parent compounds PCE, TCE, and DCE (Chu and Jewell, 1993). It is noted that anaerobic reduction of VC to ethylene is a slow and inefficient process (Freedman and Gossett, 1989). Gantzer and Wackett (1991) determined that dechlorination of chlorinated ethenes proceed via first-order rate constants.

The oxidation-reduction (redox) potential is a relatively simple and inexpensive indicator of the redox state of an aquifer. If the redox is positive, one can assume that dissolved oxygen is present and the system has not been stressed by biological

activity (Borden et al., 1995). If the redox potential is significantly negative, it can be assumed that processes favored under aerobic conditions, such as BTEX degradation, are occurring at a substantially reduced rate. Figure 7 suggests that the redox should be -400 mV or less and the dissolved oxygen should be below 0.25 mg/l before anaerobic microbial reactions could take place for the more highly chlorinated compounds (i.e., PCE, PCA). It should be stressed that one normally does not attempt to determine the actual redox conditions for comparison between different sites, rather the differences between points within a plume. Furthermore, due to the lack of internal equilibrium (Morris and Stumm, 1967) and the mixed Eh potentials of natural aqueous systems, the use of any measured master Eh as an input in equilibrium hydrogeochemical model for predicting the equilibrium chemistry of redox reactions is misleading. Instead, measuring certain sensitive species such as oxygen, Fe(II), hydrogen sulfide, or methane as qualitative guides to the redox status of the water may generate better results (Lindberg and Runnells, 1984).

In anoxic waters, where low pH and Eh exist, the reduced form of manganese, Mn(II) is favored (Stumm and Morgan, 1981). Reduction of Fe(III) and Mn(IV), due to chemical processes or microbial metabolic reactions that couple the oxidation of organic matters to the reduction of these chemical species, has a major influence on the distribution of Fe(II) and Mn(IV) in aquatic sediments, submerged soil, and ground waters (Stone and

Morgan, 1984; Burdige and Neelson, 1986; Ehrlich, 1987; Di-Ruggiero and Gounot, 1990; Lovley, 1991). Thus, measurable Fe(II) or Mn(II) may indicate suboxic conditions in the absence of detectable oxygen concentration (Higgo et al., 1996).

In addition to establishing background conditions away from the plume, dissolved oxygen, nitrate, manganese, iron, sulfate, and sulfide should be measured along the axis of the plume, as well as transverse to it, in order to characterize biological activity with respect to the redox state at those locations. This information will allow an estimation of the current redox state at various parts of the contaminated plume, thereby defining the types of reactions that may take place.

The rate of change in the concentration of these parameters can be useful as input to predictive models. This set of data will also characterize the abundance of the principal electron acceptors, oxygen, nitrate, and sulfate, to allow an estimate of how long natural attenuation will remain a viable remedial alternative.

Another approach that may be used to indicate the terminal electron acceptor process (TEAP) predominant in the areas of contamination is the hydrogen (H₂) concentration (Lovley and Goodwin, 1988). Hydrogen concentrations for the various terminal electron acceptors are shown in Table 2 (Chapelle et al., 1995).

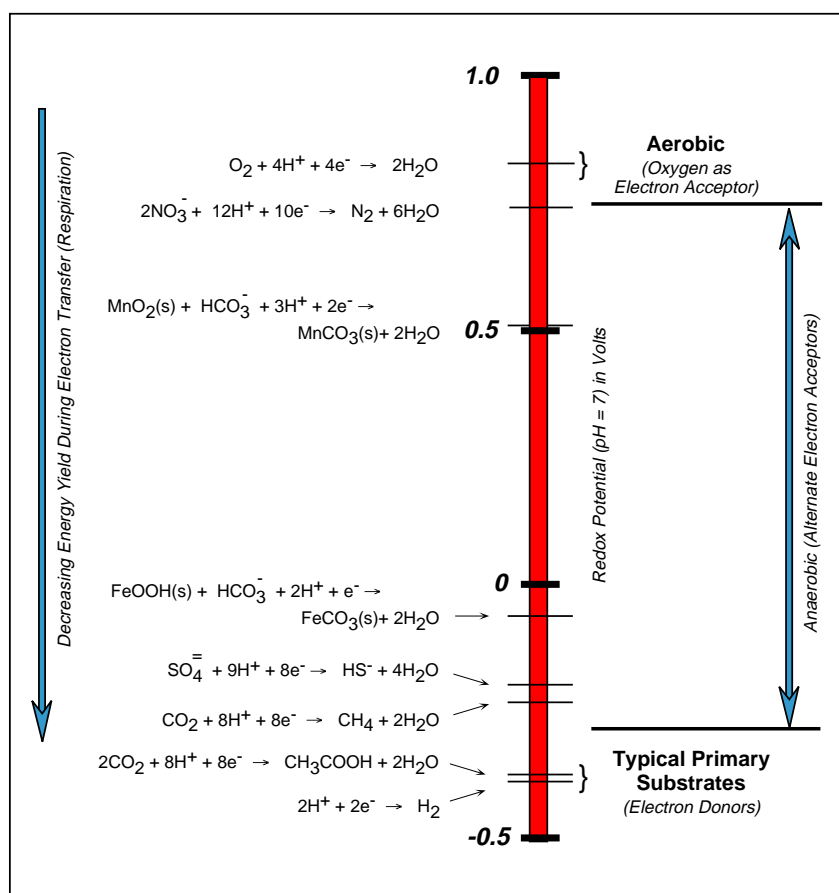


Figure 7. Important electron donors and acceptors in biotransformation processes. Redox potentials data were obtained from Stumm and Morgan (1981). (Modified from *In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies*, p. 8-3, 1993, by Norris et al., EPA/600/R-93/124.)

Table 2. Range of Hydrogen Concentrations for a Given Terminal Electron-Accepting Process. (Data from Chapelle et al., 1995.)

| Terminal Electron Accepting Process | Hydrogen (H ₂) Concentration (nanomoles per liter) |
|-------------------------------------|--|
| Denitrification | <0.1 |
| Iron (III) Reduction | 0.2 to 0.8 |
| Sulfate Reduction | 1 to 4 |
| Methanogenesis | 5-20 |

Parameters that investigators should analyze for petroleum hydrocarbons include dissolved oxygen, nitrate, Fe(II), sulfate, redox potential, pH, Mn(IV), dissolved methane, and total petroleum hydrocarbons (ASTM, 1998). The majority of these parameters can be determined using field measurements, Hach™ kits, and/or CHEMetrics™ test kits. Since methane is produced after other TEAs (nitrate, iron, sulfate) are depleted, dissolved methane data is superior to contaminant data (Underground Storage Tank Technology Update, 1998). The parameters for chlorinated solvents may include: temperature, redox potential, DO, sulfide, Fe(II), methane, ethane/ethene, alkalinity, pH, sulfate, nitrate, chloride, dissolved organic carbon, and hydrogen. Since Fe(III) may be dissolved from an aquifer matrix, Fe(II) is measured as proxy for biodegradation due to iron reduction. Although there may be a correlation between sediment redness and the hematite content of soil, when a soil sample represents a mixture of several iron species, the color is not a useful indicator (Heron et al., 1994).

The microbial activities of a site are thus determined by the dissolved organic carbon, presence of macro- and micronutrients and the TEA (Semprini et al., 1995). The presence and concentration of each will determine not only the activity, but the predominant population.

Different levels of QA/QC may be required for those analyses determined in the field versus those performed under laboratory conditions (U.S. EPA, 1996; Klusman, 1980; Shampine et al., 1992; Koterba et al., 1996). For example, dissolved iron and oxygen, redox, and temperature must be determined on-site (Shelton, 1994; Wood, 1981) using field test kits because of the deterioration that would normally occur between the time of sample collection and that of arrival at the laboratory. On the other hand, parameters such as metals, organics (Shelton and Capel, 1994; Fishman and Friedman, 1985), and bacteria can be properly preserved by cooling, capping, or chemical fixation, and thereby subjected to a higher level of QA/QC.

Factors Affecting the Demonstration of Natural Attenuation

Hydrogeology

State and federal agencies are increasingly relying on risk based corrective action (RBCA) and/or MNA for cleanup of contaminated sites (Brady et al., 1998). The American Society

for Testing and Materials released an RBCA protocol two years ago (ASTM, 1998) and recently finalized an MNA protocol. The U.S. EPA has recently published a directive on MNA (U.S. EPA, 1999) and a protocol for chlorinated solvents (U.S. EPA, 1998a). Since RBCA and MNA incorporate no safety factors to reduce contaminant concentrations compared to active remediation technologies, these strategies rely solely on accurate and high quality hydrogeological site characterizations (Boulding, 1993a and b; U.S. EPA, 1997a) to demonstrate adequate public protection.

Adequate monitoring is one of the most important facets of proving that natural bioremediation is occurring in the subsurface. Many factors other than natural attenuation or bioremediation can have an effect on the observed concentration of contaminants at a monitoring well (Black and Hall, 1984). The infiltrating precipitation into a system may have a profound effect on contaminant concentrations, especially if the contaminants are light nonaqueous phase liquids (LNAPLs) such as petroleum hydrocarbons (Kemblowski and Chiang, 1990; Lenhard and Parker, 1990). High rates of infiltration may lower the apparent concentration of contaminants due to dilution. Pettyjohn (1982) has shown that there are actually two time periods after a precipitation event when dilution may have an effect on monitoring. The first is a few hours after a precipitation event where flow is through macropores or "wormholes" to the water table, and the second is a few days after with flow through the vadose zone. The time required for both of these events to occur will be a function of the number and size of the macropores, overall permeability of the unsaturated zone and depth to the water table. Monitoring during these events can result in an apparent decrease in the concentration of contaminants by dilution, or if contaminants are present in the vadose zone, an increase in concentrations due to their infiltration. Seasonal variations may also occur in flow paths within an aquifer (Schmidt, 1977). These variations may cause an apparent increase or decrease in concentration of contaminants due to dilution or plume movement. Although dilution is considered to be a part of natural attenuation, this reduction in concentration should not be attributed to degradation.

A major problem with monitoring wells is that a sample from the uncontaminated portion of the aquifer may be a composite of contaminated water from the plume drawn into the cone of depression along with clean water from the aquifer. This will result in an apparent increase in contamination. The reverse is also often possible if the monitoring well is pumped for an extended period prior to sampling, the amount of clean water coming into the well in relation to contaminated water will result in an apparent decrease in the concentration of the contaminants of interest. These problems are often exacerbated by using well screens that are too long (greater than 2 meters), inconsistent screened intervals, and inappropriate sampling methods (Church and Granato, 1996).

Pumping wells other than those designed for monitoring (such as those in an interdiction field, or water supply wells for municipalities or irrigation) may influence the movement of a plume as well as flow lines within an aquifer. Depending on when these wells were designed and constructed, how they are pumped, and how they affect plume movement, apparent decreases in concentration may be observed in monitoring wells (Martin-Hayden and Robbins, 1997; Martin-Hayden et al., 1991; Robbins, 1989; Robbins and Martin-Hayden, 1991).

Monitoring programs should be designed such that these concerns are taken into account (Zeeb et al., 1999). A good

monitoring program will require sampling (Puls and Barcelona, 1996) of not only monitoring wells which are completed into the plume, but also monitoring wells outside of the contaminated zone in order to establish background conditions (Figure 8). Data from monitoring wells in the contaminated portion are then compared to background wells. The number and location of monitoring wells are not only determined by plume geometry and ground-water flow (ASTM, 1991), but by the degree of confidence required to statistically demonstrate that natural attenuation is taking place, to estimate the rate of attenuation processes, and to predict the time required to meet established remediation goals. These issues will be addressed in detail in the EPA guidance for long term monitoring (LTM) for MNA which is in preparation.

MNA Monitoring Well Network Considerations

RBCA and MNA both rely on sentinel wells for early warning signals of plume migration. The installation of monitoring wells (Aller et al., 1991) to adequately identify contaminant concentrations is of paramount importance in determining the overall contribution of biological processes to a reduction in either concentration or mass of contamination. Monitoring wells which are to be used to determine the contribution of natural bioremediation to site cleanup cannot be located until sufficient knowledge of the aquifer system is obtained (Zeeb et al., 1999). Information that must be obtained before installation include depth to water table, hydraulic conductivity (Molz et al., 1994) and gradient, direction of ground-water flow, storage coefficient or specific yield, vertical and horizontal conductivity distribution, direction of plume movement and the effects of any man-made or natural influences (i.e., lagoons or seeps) on the aquifer system. It is also important to determine if the hydraulic gradient is affected seasonally. Also, sentinel well screen depth and length are important. Often it is advantageous to use short screens to minimize averaging of vertical water quality differences (Martin-Hayden and Robbins, 1997).

The location, number (ASTM, 1995) and other pertinent data regarding monitoring wells (U.S. EPA, 1998b) for the evaluation of MNA should be determined on a site-specific basis. The design of the monitoring network will be determined by the size of the plume, site complexity, source strength, ground-water/

subsurface water interactions, distance to receptors and the confidence limits each party involved wishes to place in the data obtained. By necessity, the denser the monitoring network, the greater the degree of confidence one may place in the data. The wells should be capable of monitoring singular flow paths within a plume's course and subsequent movement of contaminants along these flow paths. One way to determine natural attenuation is to determine the concentration of appropriate parameters at one location and sample the same volume of water for the same parameters at some distance downgradient. It is generally impractical to monitor flow paths within a plume with the exception of the plume axis which is the only flow line that can be located with any reasonable level of certainty.

A second way to monitor for natural attenuation assumes that the plume is in equilibrium. While less costly and less time-consuming than monitoring single flow paths for contaminants where a mass balance cannot be performed, the confidence level for data obtained is significantly less when plume equilibrium is assumed. Problems arise because plumes are never in complete equilibrium. Monitoring wells even a few feet apart differ significantly in observed concentrations of contaminants. It has been suggested that internal tracers such as trimethylbenzene (TMB), a biologically recalcitrant compound, can increase the confidence one might place in this method. One simply uses the difference between TMB concentrations at the upgradient and downgradient points to measure the observed loss which can be attributed to other factors such as dispersion. One must be cautioned, however, when using TMB as a conservative tracer due to its degradation under anaerobic conditions and the resulting production of aromatic acid intermediates (Fang et al., 1997).

To monitor the anaerobic degradation of chlorinated solvents, monitoring of singular flow paths may not be necessary, if a pseudo-mass balance can be performed. This method assumes that at each monitoring point either the parent chlorinated solvent or daughter product should be observed. Using PCE as an example at each monitoring point, samples are analyzed for PCE, TCE, DCE, VC, and ethene. The total concentration of analytes is compared on a molar basis. Changes in total molar concentrations are assumed to be losses due to flaws in the monitoring system. The pseudo-mass balance method does not account for physical losses due to dispersion, sorption, or dilution, therefore, one would also have to utilize some method, such as a conservative tracer, to account for these other processes. To place even greater confidence in data, the time frame for analysis must be sufficient to allow for differences in subsurface mobility of the various chlorinated solvents. They will not arrive at the downgradient monitoring point at the same time.

Often hazardous waste sites are monitored for natural attenuation using monitoring wells which have previously been installed, especially if plume equilibrium is assumed. This in itself presents a number of problems, especially if the wells were installed without adequate knowledge of the subsurface and plume movement. If it can be demonstrated that contamination exists between these wells, by using tracer study for example, they can be used as appropriate monitoring points for assessing natural attenuation. Reinhard and Goodman (1984) used chloride as a reference to investigate the behavior of the trace organic compounds in leachate plumes of two sanitary landfills. Kampbell et al. (1995) and Wiedemeier et al. (1996) described the use of TMB to normalize for changes in BTEX concentration due to abiotic processes of dispersion, dilution, sorption, and volatilization.

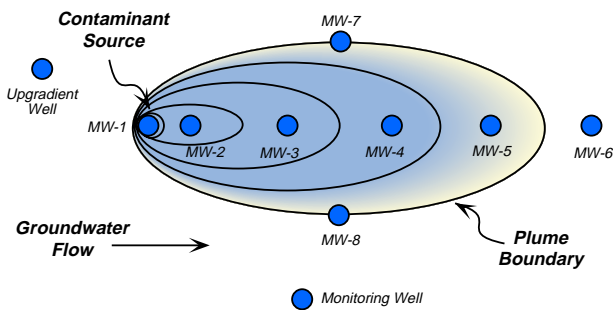


Figure 8. Recommended groundwater monitoring well network for demonstrating natural attenuation. (Reprinted from *A Practical Approach to Evaluating Natural Attenuation of Contaminants in Ground Water*, p. 166, 1994, by P.M. McAllister and C.Y. Chiang. Reprinted by permission of *Ground Water Monitoring & Remediation*, Westerville, OH, Copyright 1994. All rights reserved.)

It is also necessary to determine what contribution physical processes between sequential monitoring wells have on apparent reduction of chemicals. It may also be necessary to construct new monitoring wells which are offset to present wells or to perform borings near existing wells.

Statistical Validity of Data for MNA

Adequate monitoring is critical (Reinhard and Goodman, 1984), especially when considering natural attenuation as a remedial alternative. The number of sampling points (Barcelona et al., 1994 and 1985) and sampling rounds are often insufficient to establish statistically valid trends, given the natural variability in ground water quality, the variability due to pumping and sampling, and differences between analytical laboratories. Schmidt (1977) lists a number of conditions in which large fluctuations in water quality may be noted, indicating that minor changes in water chemistry may be related to sampling procedures. In order to minimize the effects of natural seasonal variations, sampling, and subsurface heterogeneity on ground water quality, any natural attenuation monitoring program (Gibbons, 1994; Gilbert, 1987) should be based on a detailed statistical evaluation of pertinent data (Hardin and Gilbert, 1993; McDonald and Erickson, 1994; O'Brien et al., 1991; O'Brien, 1997). Although it is recognized that intensive monitoring is expensive, increasing sampling points and frequencies, along with acceptable QA/QC procedures, will give more statistically reliable information. Caution should be exercised when drawing conclusions from limited data sets, especially when attempting to model complex situations.

The number of samples required for evaluating natural attenuation is intrinsically a function of a preselected confidence error and the variance of the data. Quarterly samples collected for a year or two, for example, may not be adequate for evaluating an overall reduction in the mass of contaminants from a particular monitoring well. Sampling frequencies such as this offer small windows for viewing contaminant reduction rates. Contaminant fronts may or may not have reached the monitoring points at the time of sampling, or as stated previously, any number of processes such as infiltration, dilution, and sorption may bias results when addressing only the bioremediation component of natural attenuation.

If the reduction of contaminant mass is to be determined by temporal trends (least squares analysis), for example, the statistical confidence is based on the variance of the data and the square root of the number of samples. In a least square analysis, the correlation coefficient (r) is a function of the degrees of freedom (df) which, in this case, is the number of observations minus two ($n-2$). For example, if a well were sampled quarterly for two years, the degrees of freedom would be 6 ($8-2$) which requires a correlation coefficient of about 0.8 to demonstrate statistical significance at a 95 percent level of confidence (an r of 1.0 denotes a perfect correlation). The point is that one must sample with enough frequency, over a protracted period in order to obtain a statistically meaningful correlation between the reduction of contaminant mass and time.

MNA Degradation Rate Constant Considerations

Precision and accuracy in estimation of rate constants are essential to conclude how quickly the ground-water plume will be cleaned up following the source control (McNabb and Doohar,

1998). Reviews by Vogel et al. (1987a) and Howard et al. (1991) contain a compilation of chlorinated solvents rate constants. Experimental data on the neutral and base-catalyzed abiotic hydrolysis rates of chlorinated ethanes and ethenes was determined by Jeffers et al. (1989). Chappelle et al. (1996) integrated field and laboratory data to estimate rates of petroleum hydrocarbon biodegradation.

In the derivation of the rate constants, it is of considerable significance to calculate concentrations at the point of compliance, compare rates at the site to those in the literature to determine if the site is behaving like other sites, and predict changes caused by fluctuations in flow (Weaver et al., 1996). Although microcosms are used as an effective tool to determine the biodegradation potentials, the use of the microbiological laboratory data for calculation of rate constants may be inappropriate since they are not always representative of the degradative rate(s) under the field conditions (U.S. EPA, 1997b). Results from laboratory studies may significantly over- or underestimate biodegradation rates if environmental conditions in the laboratory differ from conditions in the field (Borden, 1994). Rifai (1997) also points out that, although useful in evaluating the biodegradation potential, microcosms can disrupt the normal structure of ecosystems and prevent the direct extrapolation of microcosm-determined biodegradation rates to field scale. If rate constants for attenuation of chlorinated contaminants are to be used for exposure assessments, it is necessary to estimate the residence time of the contaminants in the aquifer as accurately as possible (Molz and Boman, 1997). One should also be cautioned to not substitute literature biodegradation rates in the place of site specific values.

Often, first-order kinetics obtained from field studies are used to approximate the degradation mechanism. Wiedemeier et al. (1996) described two methods to estimate first-order rate constants: (1) the use of a conservative tracer, a biologically recalcitrant compound found in the dissolved contaminant plume; and (2) the interpretation of a steady state contaminant plume as proposed by Buscheck and Alcantar (1995). The later method is founded on a one-dimensional steady state analytical solution to the advection-dispersion equation presented by Bear (1979).

Modeling as a Predictive Tool for MNA

MNA requires two types of models: (1) conceptual and (2) fate and transport. In order to understand ground water-flow and contaminant movement (Bear et al., 1992), the construction of a three-dimensional conceptual model of the site must be an integral part of any MNA Work Plan. A comprehensive conceptual model should be used as a clear and concise aid for the general understanding of the nature of the site, the acquired sampling results, and to indicate where additional sampling efforts should be directed. Once a conceptual model has been accepted, a period of monitoring is required to verify that the forecast of the conceptual model is adequate. Only when sufficient quantitative site characterization data are generated and the conceptual model is well developed, can an appropriate analytical or numerical fate and transport model be chosen for the site.

Care should be exercised when choosing the models to predict the fate and transport of contaminants in the subsurface. This may also include the use of screening models such as BIOSCREEN and BIOCHLOR (Newell et al., 1996 and 1998). Appropriateness of the model to the actual hydrogeologic

situation, assumptions, limitations, and manner of application are all considerations (Corapcioglu and Baehr, 1987; Carey et al., 1988). The overall effectiveness of the model for predicting fate and transport of contaminants at a particular site depends on all of these factors. Caution should also be exercised when drawing conclusions from limited data sets, especially when modeling complex situations. The validity of the input data is critical in determining the accuracy of predictions made with the model.

Once a model is chosen, it may be applied using the site data (Hunt et al., 1988) and calibrated. Calibration is a process of careful modification of site hydrogeologic or contaminant transport parameters over numerous simulations to identify a set of parameters which generate simulation results which closely match field measured values of hydraulic head for the flow model and contaminant concentrations for the transport model. Calibration of the model results to observed values requires that an acceptable range of error be identified for each calibration target. This range will depend on the model purpose and also on the amount and reliability of the field data (Krescec, 1997). Once calibrated, the fate and transport model can then be used to predict the future extent and concentration of a dissolved contaminant plume by simulating the combined effects of advection, dispersion, sorption, and biodegradation (Rifai et al., 1989).

Many times during calibration, if a model does not fit observed concentrations, it is assumed that the biodegradation coefficient is the proper parameter to be adjusted. Using biodegradation to adjust a model without supporting field data should not be done until all abiotic mechanisms for reduction are explored. When using a model which incorporates a biodegradation term, care should be taken to verify that assumptions made about degradation rates and the amount and activity of biomass are valid for the site in question. Degradation rates are sensitive to a wide array of field conditions which have been discussed previously. Extrapolation of laboratory derived rates to a site can also lead to significant errors. Likewise, using models to derive degradation rates from limited field data where abiotic variables are not well defined can be misleading. Models can be useful tools in determining plume movement (Mercer, 1998) and the contribution of natural attenuation (Rifai et al., 1995) to reductions in contaminant mass provided that all model inputs are correct, particularly those associated with biodegradation rates. Kinetic constants derived from laboratory microcosms or other sites are generally not useful on a wide scale to predict overall removal rates. Site specific degradation rates should be developed and incorporated into a model.

Summary

The behavior of a contamination plume, whether stable, shrinking, or expanding, is the primary evidence for the occurrence of natural attenuation. In the majority of the cases, historical data to indicate the status of a plume are not available. In these events, there are at least four basic conditions which must be present to confirm that natural attenuation processes are taking place. These include, but are probably not limited to:

1. The points of sampling must be on flow lines from the source of contamination or an upgradient point of observation. It must be demonstrated that the downgradient observations accurately reflect the abiotic and biotic processes which have occurred between

the two points. Ideally, one would sample the same volume of water at the downstream point that was sampled earlier at the source or upgradient observation well. Since this is rarely practical, it must be assumed that the plume is in equilibrium with respect to natural attenuation processes between the two points of observation. If these conditions are not satisfied, any downgradient measurement of contaminant concentration must be lower than the true value, and therefore, exaggerate the effectiveness of natural attenuation. Confidence levels may be enhanced by 1) increasing the number of observation points and times at fixed frequencies, and 2) use of a conservative tracer.

2. There must be a reduction in contaminant mass or concentration. One could argue that natural attenuation results in a reduction in contaminant concentration by sorption, volatilization, or dilution, with the only loss of mass being that of volatilization to the atmosphere. Natural bioremediation, on the other hand, must result in a reduction of mass of the contaminants of concern by the eventual conversion to environmentally acceptable compounds.
3. Site geochemistry must assure that conditions are right for reduction of contaminant concentration, such as the presence of mineral nutrients and electron acceptors, the state of redox, temperature, and pH.
4. Daughter products of contaminants must be present, perhaps with indicators of mineralization. For example, claims of intrinsic or natural bioremediation need to be supported by data including the relationship between the mass loss and the loss of oxygen. In addition to the biological utilization of oxygen, nitrate, and sulfate, these natural attenuation processes often result in the creation of by-products such as dissolved Fe(II), Mn(II), HCO_3^- , CO_3^{2-} , and methane.

The use of MNA or passive remediation at contaminated groundwater sites is gaining attention both within the scientific and regulatory communities. The OSWER directive recommends that MNA be applied concurrently with or subsequently to active measures such as source control or active remedial technologies. Selection of MNA as a remedy or part of a remedy can be advantageous since it may minimize the transfer of contaminants to other media, is less intrusive to the environment, may be applied to all or part of a site, and overall remedial costs may be lower than for an active remedy. There are several factors which may limit the application of natural attenuation as a remedial alternative. They are: (1) the longer time frame that may be required to achieve remedial goals, (2) site characterization investigations that may be more extensive and costly, (3) the added responsibility for long-term monitoring and costs, (4) toxicity of byproducts, (5) potential for continued contaminant migration, and (6) required alternatives if natural attenuation fails to meet established goals.

Under proper conditions, MNA along with source removal, long-term monitoring, and land use restrictions might be selected over more expensive conventional technologies. There will be other sites where natural attenuation will not be acceptable as a remedial alternative because of regulatory constraints or the site conditions are not favorable for its application. In the end, the selection of a remedial technology at a specific site will be

determined by time constraints in obtaining remediation objectives, the hydrogeology and geochemistry at the site, the contaminants of concern, regulatory constraints, and considerations of environmental exposure and cost.

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Quality Assurance Statement

All research projects making conclusions or recommendations based on environmentally related measurements and funded by the Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project did not involve physical measurements and as such did not require a QA plan.

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